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ON THE RELATIONSHIP OF TRISOMY 21 AND LEUKEMIA

by

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Abstract

Several disorders which have an increased incidence of malignancy have defective DNA repair. Since an increased incidence of leukemia is a feature of Down sydrome (DS), the present study was undertaken to determine whether DS cells exhibit an altered sister chromatid exchange (SCE) frequency following exposure to various mutagenic agents, as would be expected if altered DNA repair is a feature of this disorder.

Lymphocyte cultures from DS individuals, aged 3 days to 42 years, and from age and sex matched controls were exposed to 10 ug/ml of BrdU to obtain chromatid differentiation. SCE's were induced with mitomycin C (20 ng/ml) or by gamma-irradiation (200R). A minimum of 20 cells from each individual was scored for SCE's utilizing an acridine orange staining technique.

The baseline frequency of SCE's in phytohemagglutinin stimulated lymphocytes of the DS group was similar to that of controls. Addition of mitomycin C induced a significantly higher number of exchanges in the DS cells as compared to the controls. Following 200R irradiation, a similar difference between the two groups was observed.

By analogy with similar studies conducted on cells from patients with xeroderma pigmentosum (Bartram et al, 1976; Schonwald and Passarge, 1977) and Fanconi anemia (Latt et

al, 1975) it is suggested that individuals with Down syndrome may have a defect in the repair of DNA damage.

Dedication

To the memory of

Robin Elizabeth Rogers,

an angel unaware

Acknowledgements

I would like to thank Mrs. R. Nelson and Dr. J. Kuspira for introducing me to this field of study, and Mr. S. Smedstad for instilling in me the essence of the scientific method of investigation. I am grateful to Dr. P. Bowen from whom I gained a knowledge of cytogenetics and a critical and succinct method of manuscript writing. I am indebted to Mrs. F. Williams for providing her excellent technical expertise. In addition, I express my thanks to K.G., R.M.B., P.D.O., A.W., the M.B.'s, G.L.D. and C.C. for helping me to choose a path with a heart. Finally, I would like to thank my wife, Lynn, for her support and companionship throughout this endeavour.

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I. Introduction

Although clinicians have been aware of the simultaneous occurrence of Down syndrome (trisomy 21) and leukemia since the early half of the present century, it is only more recently that this association has been recognized as being more frequent than would be expected by chance. Earlier theories explained this occurrence as resulting from a common teratogenic influence; however, knowledge of the aneuploid etiology of Down syndrome made it apparent that Down syndrome was a disorder of multiple primary anomalies which had as one of its features, an increased incidence of leukemia.

An increased incidence of leukemia is also associated with some rare monogenic disorders such as ataxia telangiectasia, Bloom syndrome and Fanconi anemia. These disorders are also characterized by _in vitro_ chromosomal instability (German, 1972). When cells from these patients are cultured, they exhibit a higher frequency of spontaneous chromosome aberrations (chromatid breaks, chromosome breaks and quadriradial configurations) when compared to control cultures. For this reason, these disorders have been termed "chromosomal breakage syndromes".

Trisomy of chromosome 21 also produces chromosome instability in the affected individual; however, in this instance, the instability is not spontaneous in that it is

only observed following treatment of the cells with various mutagenic agents. For example, if cells with trisomy 21 are irradiated, then one observes an increased frequency of chromosomal aberrations as compared to controls cells treated similarly (Sasaki and Tonomura, 1969).

Recently, more sensitive methods have been developed to detect the mutagenicity of various agents at the chromosome level (see Beek and Obe, 1975). Therefore, the present study was initiated to determine:

1. Whether previous studies on trisomy 21 cells, utilizing gross chromosome breakage events, could be confirmed using a more sensitive indicator of chromosome damage, the sister chromatid exchange test. Furthermore, in view of the mentioned similarities between Down syndrome and the "chromosomal breakage syndromes", the present study may shed light on,
2. Whether the increased incidence of leukemia in Down syndrome individuals could be a consequence of defective DNA repair as indirectly assessed by the sister chromatid exchange test.

II. Literature Review

A. Etiology of Down syndrome

In 1866, Langdon Down reported his observations on the ethnic classification of idiots stating that " a large number of congenital idiots are typical mongols"(Smith, 1976). Mongoloids or mongoloid idiots, as these individuals were first referred to, are now known as Down's Syndrome, or more appropriately, Down syndrome individuals. [1]

Waardenburg (1932) suggested a chromosome aberration as a possible mechanism of Down syndrome. Penrose (1939) arrived at the same conclusion stating that " mongolism and some other malformations may have their origins in chromosome abnormalities." This hypothesis was first tested in 1952 when Mittwoch studied meiosis in a male patient with Down syndrome and found 24 chromosome bodies in the spermatocytes. She concluded from this that there was no abnormality of the chromosomes in Down syndrome and that the chromosome number of the human organism was 48, as had been

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[1] The terms mongol or mongolian idiot were originally used by Langdon Down (1866). In 1966, Allen and a number of other investigators suggested that these terms should be dropped since they implied undesirable racial connotations. They suggested as an alternative "the Langdon-Down anomaly", "Down's anomaly", "Down's Syndrome" or "congenital acromicria." Although mongolism persisted in the literature, many authors adopted this suggestion. Recently it has been recommended that the non-possessive sense of the term be used, hence the designation Down syndrome (Smith, 1975).

suggested by earlier work (see Painter, 1923; Hamerton, 1971). In 1956, Tjio and Levan established the normal human diploid number at 46 and it was shortly thereafter that Lejeune (1959) demonstrated that Down syndrome individuals have an extra, small acrocentric chromosome, trisomy 21-22. More recently both the clinical and cytogenetic features of this disorder have been thoroughly reviewed (Hall, 1964; Penrose and Smith, 1966 and Hamerton, 1971).

B. Association of Down syndrome and Leukemia

One of the many interesting observations that have been made regarding Down syndrome is that the frequency with which this condition is associated with malignancy, particularly leukemia, is greater than would be expected by chance. Brewster and Cannon first reported a case of Down syndrome associated with leukemia in 1930. Subsequent case reports by several investigators (Bernard et al, 1954;Schunk and Lehman, 1954; Carter, 1956; Krivit and Good, 1956 and Merrit and Harris, 1956) appeared to confirm this association.

In a nationwide survey carried out in the United States, Krivit and Good (1957) found 34 cases of leukemia associated with Down syndrome in the 0-4 year age group ascertained between 1952-55. This was compared with a liberal estimate of 12.3 expected cases. In other words they

found that the "incidence of joint expression of these diseases was approximately three times that anticipated." They also reported that the frequency of congenital leukemia among newborn Down syndrome individuals was nine times that of non-affected individuals.

Stewart et al (1958), in a retrospective study of childhood malignancy in Great Britain, found 17 cases of Down syndrome associated with leukemia in the 0-10 year age group ascertained between 1953-1955. They suggested that the incidence of this association was 20 times that expected and concluded that "the association evidently is not a fortuitous one." Tough et al (1961), utilizing the data of Stewart et al (1958), remarked that the frequency of Down syndrome at birth is about 1/600 (0.17%) and if one assumes that all these individuals survive, the risk of children with Down syndrome developing leukemia was a minimum of 15 times the incidence in the general population. Considering the high childhood mortality rate for Down syndrome, Tough et al (1961) concluded that "the liability of mongols to develop leukemia may be appreciably greater than that...(15 times)".

Perhaps the most convincing study was reported by Holland et al (1962) concerning a prospective study of 2033 individuals with Down syndrome for periods ranging from 1-14 years. During the period of study, 14 individuals died of malignancy, 7 of which were leukemia. They concluded that the death rate from leukemia was 18 times greater in the

Down syndrome population as compared to the general population. In view of these observations, Hamerton (1971) has concluded that " there can be no doubt that the frequency with which Down's Syndrome and leukemia are associated is considerably greater than would be expected by chance."

There is also evidence to suggest that individuals with Down syndrome are prone to develop other forms of malignancy in addition to leukemia. Holland et al (1962), found that patients with Down syndrome died from other types of cancer 2.6 times more frequently than controls. Miller (1966) has suggested that individuals with Down syndrome may be particularly prone to develop central nervous system tumours, although in a subsequent article (Miller, 1970) he stated that "the numbers are small and may well reflect chance occurrences." In summary, Down syndrome may be regarded as a constitutional chromosome abnormality which has an associated predisposition to the development of malignancy in general, although a certain neoplastic state (in this case leukemia) is more likely to develop than other forms.

C. Hypotheses Regarding the Simultaneous Occurrence of Down syndrome and Leukemia

Two major hypotheses have been advanced to explain the simultaneous occurrence of Down syndrome and leukemia. An early view held that some teratogen acting at a specific developmental stage, may result in both diseases (see Merrit and Harris, 1956 and Stewart et al, 1958 below). On the other hand, the combined occurrence of these two disorders may reflect an increased susceptibility of Down syndrome children to the development of leukemia.

In 1956, Merrit and Harris proposed a so-called "common teratogen" theory, by suggesting that "the stress which injures the fetus at the six- to nine-week period in Mongolism may also injure the developing bone marrow and the hematopoietic system in such a way as to result in leukemia at a later date." In 1958, Stewart and her co-workers arrived at a similar conclusion, that is, "it is therefore more likely that the two diseases are influenced by a common factor than that the antecedent condition, mongolism, predisposes to the later condition, leukemia." They found increased maternal age to be one such "common denominator."

In 1959, Lejeune showed that Down syndrome was a consequence of trisomy of chromosome 21, thus invalidating Merrit and Harris's (and by inference Stewart et al's) basic assumption that a "stress (physical, chemical, infectious or a fortuitous combination of these) is placed on the

developing organism and produces Mongolism." It now became apparent that a constitutional chromosome abnormality, namely trisomy 21, somehow increased one's risk of developing leukemia.

In 1961, Tough et al suggested that chromosome 21 carried loci which affect leukopoiesis. This conclusion was based on the observation that neutrophils in Down syndrome individuals have reduced lobulation indicating relative immaturity of these cells (Mittwoch, 1957). They also suggested that anomalies at these leukopoietic loci could lead to leukemia. Therefore, Down syndrome individuals would be predisposed to leukemic change because the aneuploid condition upset the normal leukopoietic mechanism.

Ross et al (1963) expanded this hypothesis by suggesting that children with Down syndrome have an "intracellular defect in the regulation of the production or maturation of the leukocytes" and that this "labile granulopoiesis" results in these children being "more susceptible to the factor or factors which result in true leukemia." Unlike Tough et al, these authors postulated that the leukemia was a combined consequence of an intrinsic defect which made these children more sensitive to extrinsic environmental carcinogens. Although this hypothesis may account for the increased incidence of leukemia, it fails to explain the increased incidence of other malignancies that has been reported (Holland et al, 1962).

Several more recent observations appear to confirm this

"increased carcinogen susceptibility" hypothesis. It has been observed that cells from these individuals exhibit an increased frequency of chromosome aberrations following viral infection (Todaro and Martin, 1967; Higurashi et al, 1973; Potter and Potter, 1975), treatment with chemical mutagens (O'Brien et al, 1971; Schuler et al, 1972) or X-ray irradiation (Sasaki and Tonomura, 1969; Higurashi and Conen, 1973; Holmberg, 1974). Since chromosome aberrations are now considered to be important from both an etiologic and prognostic point of view for certain malignancies (see Mitelman et al, 1972 and 1976, and Rowley, 1973-1976), these observations lend support to the hypothesis that the increased incidence of leukemia in Down syndrome individuals is a result of an increased susceptibility to various biological, chemical and physical oncogenic agents.

This increased susceptibility to various oncogenic agents could theoretically be the result of several mechanisms. Since most oncogenic agents produce their effect by direct damage to DNA, altered DNA repair systems may play an important oncogenic role. On the other hand, it may be that these individuals have, as a result of chromosome imbalance, other abnormal features which make malignancy more probable. For example, in Down syndrome there is evidence of altered immunologic function (Levin et al, 1975) which could mean that surveillance directed against potential cancer cells is inadequate (Burnet, 1970).

The present study was focused on the former hypothesis,

that is, the increased incidence of leukemia in children with Down syndrome may result from inadequate repair of damaged DNA. In the study an indirect method of studying DNA repair was utilized--the sister chromatid exchange (SCE) test (Beek and Obe, 1975).

D. Sister Chromatid Exchanges:Relation to DNA Repair

Sister chromatid exchanges (SCE), defined as, exchanges of homologous regions of a chromosome between sister chromatids of a metaphase chromosome, were first detected by Taylor et al (1957, 1958) in Vicia using 3-H-Thymidine incorporation and autoradiography. Subsequently, this phenomenon was found in other species (Peacock, 1963; Marin and Prescott, 1964; Walen, 1965) so that it appears to be a universal process.

Until recently, studies of this phenomena have been limited primarily because autoradiography is time-consuming and lacks a certain degree of precision. Recent techniques have made it possible to distinguish sister chromatids from one another without using autoradiography. These techniques are based on the substitution of thymidine by 5-bromodeoxyuridine (BrdU) in two successive S phases of the cell cycle. This results in the substitution of thymidine in both strands of DNA in one chromatid while the sister chromatid is substituted in only one strand of DNA. The

bifilarly substituted DNA shows a faint and the unifilarly substituted DNA a bright fluorescence after staining with Hoechst 33258 (Latt, 1973) or Acridine Orange (Dutrillaux et al, 1974; Kato, 1974a). BrdU substituted chromatids also stain differentially with Giemsa after prestaining with a fluorescent dye and light exposure (Kim, 1974; Perry and Wolff, 1974; Biederman and Bowen, 1976) or following denaturing procedures omitting the fluorescent staining step (Korenberg and Freedlander, 1974). In all of the above mentioned techniques, SCE are seen as switches in the intensity of the stain from one chromatid to the other (Fig. 1).

Since the discovery of these exchanges, there has been considerable debate over their nature, i.e., whether they represent spontaneous or induced phenomenona (Taylor et al, 1958; Marin and Prescott, 1964; Wolff, 1964). Recent evidence suggests that at least some of these exchanges are induced, since the rate of formation of SCE is dependent upon the amount of tritiated thymidine and (to a lesser extent) BrdU that has been incorporated (Gibson and Prescott, 1972; Wolff and Perry, 1974).

Several studies have shown that the distribution of SCE between chromosomes in the complement is a function of the chromosome length (Herreros and Gianelli, 1967;Latt, 1974a; Galloway and Evans, 1975; Smyth and Evans, 1976

Figure 1. Human metaphase chromosomes treated to distinguish sister chromatids from one another (see text); sister chromatid exchanges are seen as discontinuities in the intensity of staining (i). Discontinuities such as (ii) result from "chromatid twisting" and do not represent true exchanges. This metaphase spread was initially stained with acridine orange (a), followed by Giemsa (b), demonstrating that the bright fluorescent chromatid corresponds to the dark chromatid of the Giemsa preparation.

and Crossen et al, 1977). These observations suggest that the interchromosomal location of any given exchange is a function of chance alone. In other words, SCE's have an equal chance of occurring anywhere in the complement, uninfluenced by the location of other exchanges.

Although the interchromosomal location of these exchanges is randomly determined, the intrachromosomal distribution is uneven. In some species there is an overrepresentation in the juxtacentromeric regions, although this is not the case in humans (Smyth and Evans, 1976). In human cells, the majority of BrdU exchanges occur in the midarm regions, specifically in the interband regions, as defined by quinacrine fluorescence (Latt, 1974a) or Giemsa (Crossen et al, 1977) banding. These observations lend support to Dutrillaux's hypothesis (Dutrillaux et al, 1977) that the interband regions of chromosomes "should be considered as the fragile sites of chromatids."

The finding that the frequency of SCE's can be increased by various agents which damage DNA including X-ray irradiation (Marin and Prescott, 1964; Gatti and Oliveri, 1973; Gatti et al, 1974), UV-light (Kato, 1973; Rommelaere et al, 1973; Wolff et al, 1974) or treatment with certain chemical mutagens, for example mitomycin C, methylmethane sulphonate, cyclophosphamide, bleomycin and trenimon (Latt 1974b; Kato, 1974b; Beek and Obe, 1975; Perry and Evans, 1975; Vogel and Bauknecht, 1976) suggests that SCE's may reflect repair mechanisms involved in the repair of DNA

damaged by these agents (Fig. 2).

Kato (1973) first suggested a relationship between SCE's and DNA repair as a result of finding that caffeine, a known inhibitor of postreplication repair (Rauth, 1967; Fabre, 1972), reduced the number of U.V.-light induced exchanges. He subsequently demonstrated a similar reduction of SCE's induced by 4-nitroquinoline 1-oxide and mitomycin C (Kato, 1974b). Bender et al (1974) postulate that SCE's may be related to recombinational repair. Bender et al (1974) noted that:

1. Both SCE's and recombinational repair are stimulated by UV-light, and
2. Both are inhibited by caffeine.

Other models which suggest that SCE's reflect DNA repair processes have been proposed by Latt (1974b) and Comings (1975).

Recently, Shafer (1977) has proposed a replication (in contrast to a post-replication) bypass model of SCE's adding that "it seems unlikely that SCE's can be identified as the product of known repair processes." This last statement is based on the observation that patients with xeroderma pigmentosum (a condition with deficient excision or in some cases, post-replication repair) do not show any changes in baseline SCE frequencies (Wolff et al, 1975; Kato and Stich, 1976). However, Passarge and coworkers (Bartram et al, 1976; Schonwald and Passarge, 1977) recently demonstrated that if cells from patients with xeroderma pigmentosum are stressed

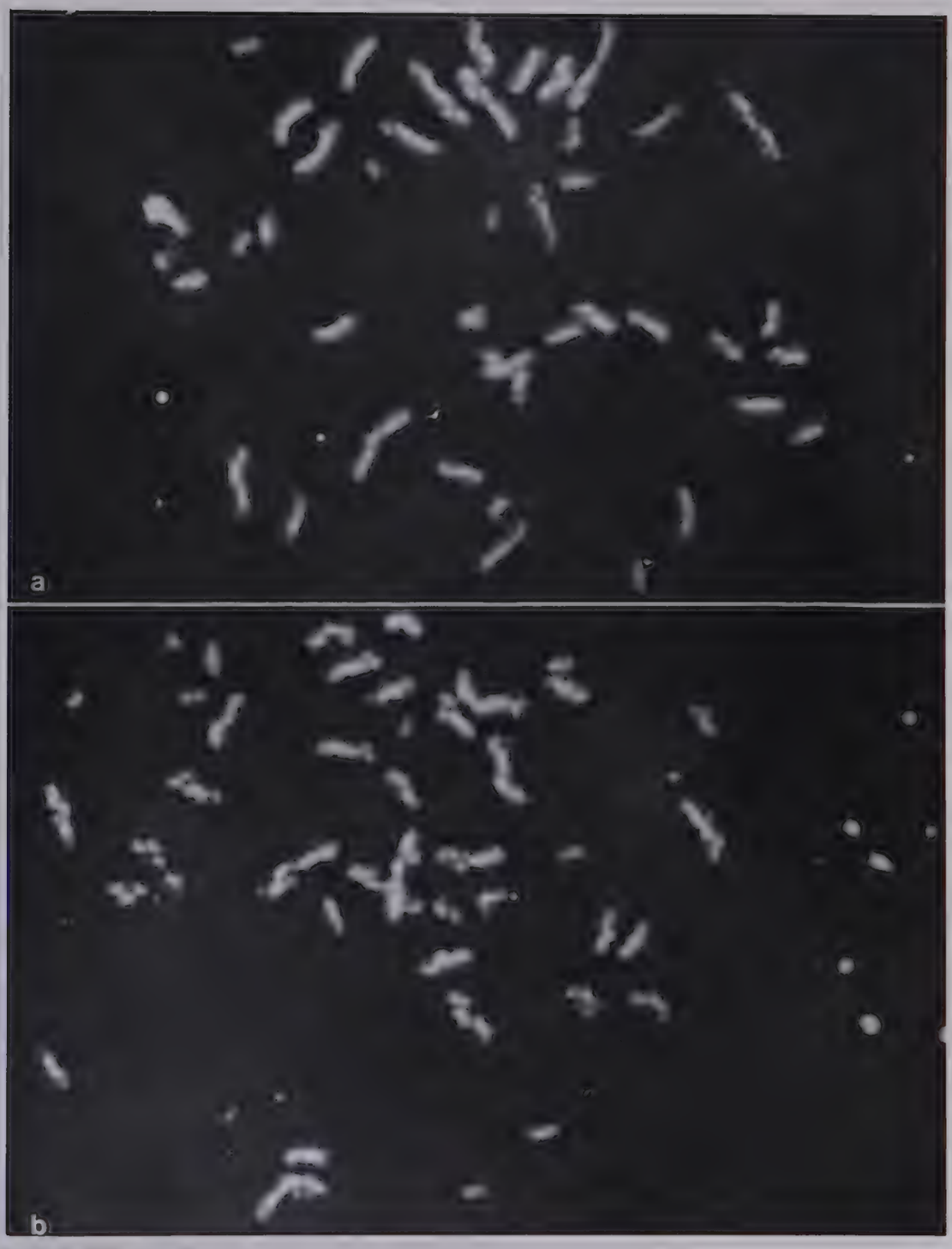

Figure 2. Sister chromatid exchanges from cultures grown under identical conditions to Fig. 1 with the exception that Mitomycin C was added at a concentration of 20 ng/ml. The upper figure (a) is from a control while the lower figure (b) is from a Down syndrome culture. Note the higher frequency of SCE's in the lower figure.

(with UV light) then the SCE frequency is significantly higher than similarly treated controls. They concluded that "the induction of SCE by ultraviolet light in xeroderma pigmentosum lymphocytes can be readily explained by the defective excision of U.V.-induced pyrimidine dimers from DNA." In other words, when these cells are stressed with U.V. light, they are unable to repair the damage prior to replication (as a consequence of defective repair of U.V.-induced damage); however, following replication, the damage is repaired by a post-replication repair system (which presumably involves exchanges between sister chromatids).

E. DNA repair: Relationship to Neoplasia

Carcinogenesis, or the "production of a cancer", is generally thought to consist of two processes--initiation and promotion (Berenblum, 1954). Considering initiation, this can apparently be a consequence of at least two different mechanisms, gene derepression or gene mutation. The second of these is important in considering the role of DNA repair to carcinogenesis.

Presumably, during initiation, a carcinogen (physical, chemical or biological), interacts with DNA, in some instances damaging it to a variable degree. There is some evidence to suggest that this damage is agent specific

(Mitelman et al, 1972, Rowley, 1974); that is, some oncogenic agents produce specific, non-random chromosome changes.

This altered DNA may result in one of three consequences. Firstly, the damage may be repaired by a repair system restoring the DNA back to its original structure. On the other hand, if repair is defective (either qualitative or quantiative) then a mutation may result. Finally, this altered DNA may result in a re-awakening of dormant genes (gene derepression). The second consequence, namely inadequate DNA repair resulting in a mutation, may result in tumour formation according to the "somatic mutation theory of carcinogenesis".

The somatic mutation theory of carcinogenesis was initially proposed (Bauer, 1928) to account for the observation that the neoplastic properties of a given cell are transmitted to the daughter cells following cell division. Thus, cancer was viewed as a process intimately involved with the genetic material of the cell. It is now presumed that the mutation which results in the malignancy, affects genes which are involved in the regulatory control of normal cell growth (Friedberg, 1976). Therefore, defective DNA repair may directly contribute to the initiation of malignancy (somatic mutation theory of carcinogensis), or it may indirectly contribute by increasing chromosome instability (German, 1972).

As mentioned previously, at least two clinical

disorders with known defects in the repair of DNA, are associated with an increased predisposition to malignancy. Xeroderma pigmentosum, a disorder associated with a high incidence of skin malignancies (Robbins et al, 1974) has been shown to result from defective ultraviolet-stimulated repair replication (Cleaver, 1969). Ataxia telangiectasia, a disorder associated with lymphoreticular neoplasia, has recently been shown to be defective in the excision repair of gamma-ray damaged DNA (Paterson et al, 1976).

III. Materials and Method

A. Patients and Controls

Twenty-one patients with cytogentically verified complete trisomy 21, whose ages ranged from 3 days to 28 years, were involved in the study. These individuals were either patients at the University of Alberta Hospital or residents of Winnifred Stewart School or the Western Industrial Research and Training Center. Healthy controls were matched for both age and sex. Due to culture failures, only ten pairs were analysed for the baseline study, and five pairs were analysed for the induction studies.

B. Lymphocyte cultures and chromosome preparation

Blood samples were collected in a heparinized vacutainer by venipuncture. Approximately 0.25 ml of whole blood was added to a sterile prescription bottle containg 4 ml. of Medium 199 and 1 ml. of fetal calf serum. Penicillin and streptomycin were present in a final concentration of 100 units and 100 ug/ml., respectively, to prevent bacterial contamination. In addition, BrdU (5-bromodeoxyuridine; Sigma, St. Louis) was added to each culture in a concentration of 10 ug/ml. The cultures were shielded from

light (by using a black cloth bag), since it had been previously shown that photolysis of BrdU containing chromatids enhances the sister chromatid exchange frequency (Ikushima and Wolff, 1974). Phytohemaglutinin (Difco, Detroit) was used as a mitotic stimulant.

Cultures were incubated at 37.0°C for 72 hours. Colcemid (Gibco, New York) was added to each culture at a concentration of 10 ug/ml. of medium during the terminal three hours. The cultures were then transferred to 15 ml. centrifugation tubes and spun at 600-800 RPM for 10 minutes. The supernatant was removed and prewarmed hypotonic solution (0.075 M KCl with 16 units/ml of heparin) was added. This suspension was placed in a waterbath (37°C) for 10 minutes to facilitate chromosome dispersal. The suspension was then respun at 600 RPM for 10 minutes, following which the hypotonic solution was replaced with cold fixative (3:1 methanol/glacial acetic acid at 4°C). The fixative was removed and replaced with fresh fixative twice.

Air dried slide preparations (one drop of cell suspension pipetted onto a clean slide) were coded to eliminate observer bias, then stained in acridine orange (50 ug/ml. in distilled water). Optimal differentiation of chromatids was obtained if the slides were stained for 2-3 minutes followed by rinsing in distilled water.

Sister chromatid exchanges were scored, utilizing ultraviolet light fluorescence microscopy (Leitz Orthoplan with 100 W mercury lamp, excitor filter BG 38 and barrier

filter TK 496). Whenever possible, a minimum of 20 cells was examined and a mean frequency of exchanges per cell was computed. Exchanges occurring at the centromeric region were not scored since in second division mitoses , it is difficult to distinguish between chromatid twisting and true exchanges (Fig. 1).

C. Exposure to mitomycin C and gamma-rays

For the cultures in which mitomycin C was added, this agent was present throughout the culturing period in a concentration of 20 ng/ml. of culture medium Mitomycin C was kindly donated by Dr. P. Bland. The gamma rays were produced by a Gammacell 40 at a dose rate of 101.2 R/min. The use of this instrument was kindly provided by Dr. T. Wegman of the Dept. of Immunology. Cultures were irradiated with 200 R, 48 hours after initiation of the respective cultures.

D. Experimental Design

All cultures from each individual were set up in duplicate from a single blood specimen. Controls were handled at the same time and the same manner as the test cultures. The baseline frequency cultures were handled at the same time and same manner as the induction cultures. In other words, a single run of the experiment (one Down syndrome sample and one control specimen) would consist of setting up a minimum of six cultures from each individual making a total of twelve cultures.

E. Statistical analyses

Variation in the SCE frequency of the two groups, as caused by mitomycin C and gamma-irradiation, was tested statistically using the "paired t-test." Computations were facilitated with the use of an automatic desk computer.

IV. Results

Tables 1, 2 and 3 summarize the results obtained. When no mutagenic agent is present in the culture, the SCE frequency of Down syndrome lymphocytes is comparable to that of simultaneously cultured controls (Table 1). The mean frequency of SCE's for the control group was 6.17 exchanges per cell, while that of the Down syndrome group was 6.54. There is no statistically significant difference between the two groups as determined by the paired t-test. The "baseline frequency" reported here is comparable to that of other published studies. For example, Crossen et al (1977) reported a mean frequency of 6.37 exchanges per cell in 20 normal subjects. Other studies, for example Gallowaway and Evans (1975), have reported higher baseline frequencies of SCE's in normal subjects. These differences may be partly explained by differences in the culturing media used, and differing concentrations of BrdU. In addition, some studies have included centromeric exchanges in the computation of the SCE per cell, while others have not included these.

When mitomycin C is present, the SCE frequency is elevated. For the controls, a mean value of 24.12 exchanges per cell was observed. This value is also comparable to other studies (see Fig. 5 in Latt, 1974b). For the Down syndrome group, the mean value was 35.10, which is significantly different from the control result. In other

words, following exposure to Mitomycin C, Down syndrome lymphocytes exhibit a significantly higher frequency of SCE per cell, as compared to simultaneously cultured control cells. If the increased frequency of exchanges is divided by the respective pretreatment baseline (to obtain a relative increase in exchanges), the response of the Down syndrome individuals is 1.35 that of normals. In addition to the increased frequency of SCE, the Down syndrome metaphases also exhibited a higher frequency of chromosome aberrations, although this was not quantitated in the present study.

Table 3 compares the results obtained following gamma-irradiation. The Down syndrome lymphocytes exhibit an increased SCE frequency as compared to control cells. In this instance, the difference is not as great as that obtained with mitomycin C (Fig. 3). There was also an increase in chromosome aberrations and a decrease in the number of second division mitoses in the Down syndrome cultures as compared to controls following gamma-irradiation. This is interpreted as another indication of the increased radiosensitivity of Down syndrome cells.

TABLE 1. BASELINE FREQUENCY OF SISTER CHROMATID EXCHANGES IN CULTURED LYMPHOCYTES

	CONTROL		DOWN SYNDROME	
	MEAN	ST.DEV.	MEAN	ST.DEV.
I	4.95	2.15	6.73	2.57
II	7.05	2.70	7.15	3.06
III	7.87	3.63	9.00	3.91
IV	6.43	2.10	7.00	2.79
V	5.40	1.91	5.61	1.99
VI	5.12	1.94	5.88	2.57
VII	7.25	3.08	6.27	3.45
VIII	5.40	1.85	5.26	2.10
IX	6.01	1.45	6.25	1.29
X	6.32	2.98	6.22	2.29

$t=1.49,\ 0.20 > p > 0.10$

TABLE 2. SISTER CHROMATID EXCHANGE FREQUENCY FOLLOWING TREATMENT WITH MITOMYCIN C (20 ng/ml)

	CONTROL		DOWN SYNDROME	
	MEAN	ST.DEV.	MEAN	ST.DEV.
III	23.00	6.70	32.56	8.25
V	28.38	5.54	38.70	6.85
VI	28.40	5.59	44.47	6.19
VII	19.35	3.41	26.16	4.20
XI	21.45	4.65	33.60	5.60

$t=7.15$, $p < 0.01$

TABLE 3. SISTER CHROMATID EXCHANGE FREQUENCY FOLLOWING TREATMENT WITH GAMMA-IRRADIATION (200R)

	CONTROL		DOWN SYNDROME	
	MEAN	ST.DEV.	MEAN	ST.DEV.
VII	9.36	2.97	13.12	5.07
VIII	8.40	2.22	13.91	2.64
IX	8.60	2.91	11.85	2.68
XI	8.53	1.39	11.79	1.80
XII	8.62	2.42	13.10	3.18

$t=9.47$, $p < 0.01$

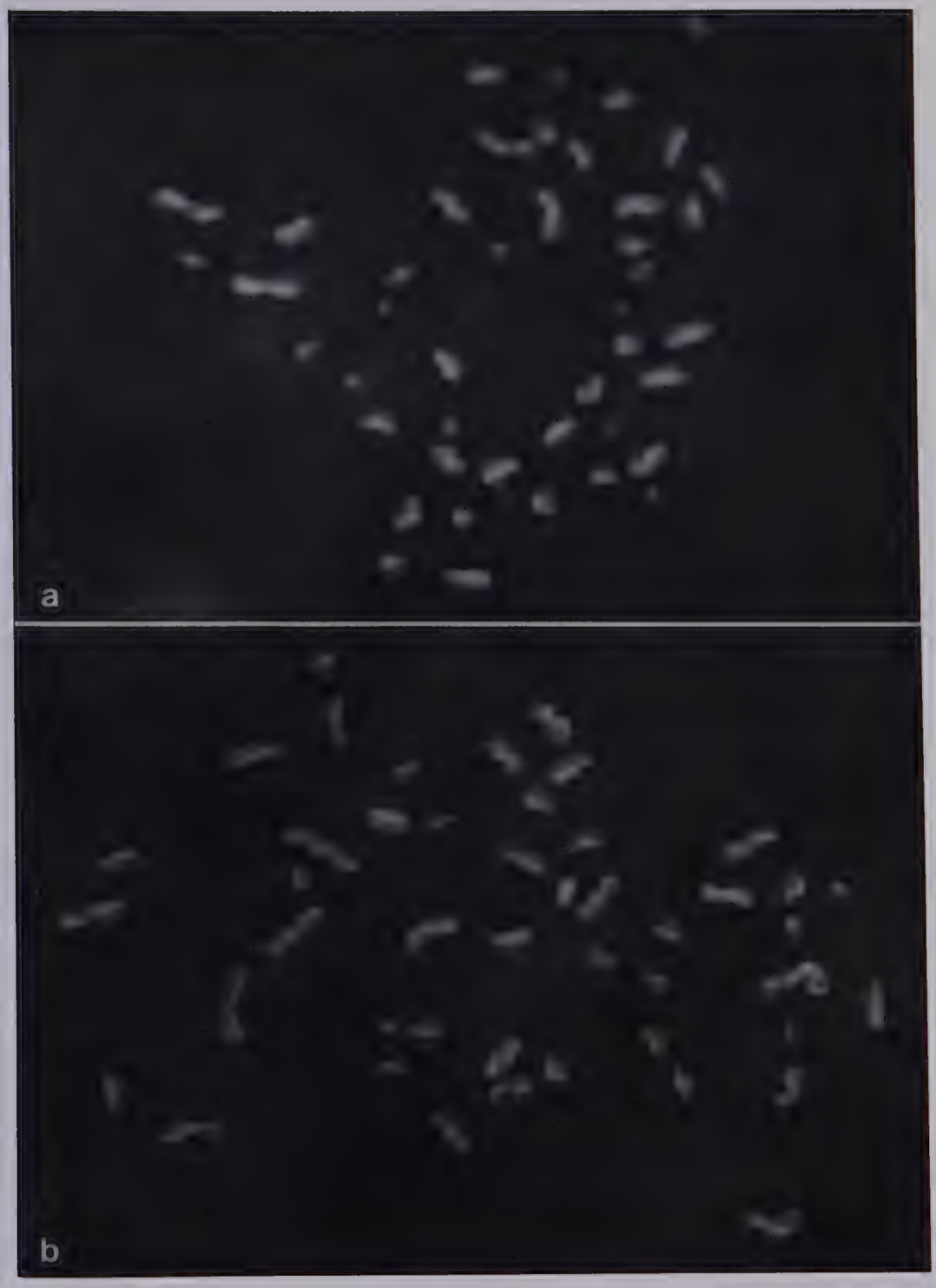

Figure 3. Comparison of SCE frequency following gamma irradiation in control (a) and Down syndrome (b) culture. Note that the observed difference is not as great as the Mitomycin C treatment (Fig. 2).

V. Discussion

There are two general possibilities concerning the relationship between the increased frequency of SCE's in trisomy 21 cells and the increased incidence of malignancy. These may both be a result of some common etiologic factor (for example, as a result of defective DNA repair), or the first observation may reflect some other process which may not be related to the increased incidence of malignancy.

If the relationship is a direct one, then one should find similar studies which have drawn this same conclusion. Latt et al (1975) studied SCE frequency in lymphocytes and fibroblasts from patients with Fanconi Anemia, both a baseline level and an induced level, produced by mitomycin C (see Table 4). They observed that cells from the normal group had a higher SCE frequency following exposure to mitomycin C than did the Fanconi Anemia group. They suggested that "the chromosomal breaks and rearrangements in Fanconi's anemia lymphocytes may result from a defect in a form of repair of DNA damage."

Recently Schonwald and Passarge (1977) reported a similar study conducted on patients with xeroderma pigmentosum (see Table 4). Following exposure to U.V. light, cells from six patients showed an increased rate of sister chromatid exchanges as compared to simultaneously cultured controls. They concluded that this finding was "an

TABLE 4. COMPARISON OF THE PRESENT STUDY TO SIMILAR STUDIES DONE ON PATIENTS WITH KNOWN DEFECTIVE DNA REPAIR MECHANISMS

DISORDER	TREATMENT	SCE FREQUENCY
Fanconi anemia	Mitomycin C	control>FA[1]
Xeroderma pigmentosum	U.V. light	XP>controls[2]
Down syndrome	Mitomycin C Gamma-rays	DS>controls[3]

LEGEND

SCE - Sister chromatid exchange

FA - Fanconi anemia

XP - Xeroderma pigmentosum

DS - Down syndrome

References

(1) Latt et al, 1975

(2) Bartram et al, 1976 and Schonwald and Passarge, 1977

(3) Present study

expression of U.V. light induced chromosomal instability due to impaired DNA repair."

When compared to the study of Schonwald and Passarge (1977, Table 4), one could suggest that the results of the present study are consistent with the interpretation that cells from individuals with Down syndrome have impaired DNA repair in response to gamma ray and mitomycin C insult, and that this may partly explain the increased incidence of malignancy that this disorder shares with xeroderma pigmentosum and ataxia telangiectasia.

Since the completion of the present study, two reports have appeared which lend support to the present thesis. Countryman et al (1977) have suggested that the increased aberration frequency observed in Down syndrome cells is a consequence of competition between a (hypothetical) error-free repair system and an error-prone (?post-replication repair) system that generates chromosome aberrations. Presumably, Down syndrome cells use this "error-prone" system to a greater extent than controls. One wonders if this "error-prone" mechanism is in fact the post-replication repair system referred to earlier. Furthermore, on the basis of finding that cells from patients with Down syndrome have decreased U.V.-light induced repair synthesis, Lambert et al (1976) have concluded that "DNA repair mechanisms are impaired in leukocytes from patients with Down's syndrome, which may contribute to the increased incidence of leukemia and the

susceptibility to X-ray irradiation in this disorder."

An alternative explanation of the present study would be that the increased chromosome aberrations (including the SCE frequency), as well as the increased incidence of malignancy is a result not of defective repair of DNA damage but as a consequence of an overload of the repair systems due to a reorientation of the so-called "bodyguard layer of constitutive heterochromatin" (Hsu, 1975). According to this hypothesis, constitutive heterochromatin may be used by the cell as a bodyguard to protect the very important euchromatin by forming a layer of dispensible shield on the outer surface of the nucleus. Mutagens, clastogens or even viruses attacking the nucleus must first contact this bodyguard layer which absorbs the assault, thus sparing the euchromatin regions from damage. Since Down syndrome cells exhibit an increased frequency of chromosome aberrations (see earlier references), or an elevated SCE frequency (present study) after X-ray irradiation, treatment with chemical mutagens or viral infections, it is tempting to speculate that the trisomic state may somehow alter this "bodyguard layer", making it less effective. Although several investigators have reported normal constitutive heterochromatin banding patterns in metaphase chromosomes from patients with Down sydrome (Chernay et al, 1971; Ridler, 1971; Pathak and Sinha, 1972), this hypothesis would be best tested by studying interphase nuclei.

In conclusion, sister chromatid exchanges were studied

in lymphocytes of patients with Down syndrome in an attempt to better understand the increased incidence of malignacy that has been observed for patients with this constitutional chromosome abnormality. By comparison with similar studies conducted on cells from patients with xeroderma pigmentosum (Bartram et al, 1976; Schonwald and Passarge, 1977) and Fanconi anemia (Latt et al, 1975), it is suggested that patients with Down syndrome may have a defect in the repair of DNA damage and that this may directly or indirectly contribute to the increased incidence of malignancy that has been observed in these children. Furthermore, this defective DNA repair would presumably be a consequence of the aneuploid state interfering with the normal repair mechanisms. This interference may be a result of a gene dosage effect for genes contolling the normal DNA repair processes. In other words, chromosome 21 may carry genes which regulate or are somehow involved with normal DNA repair systems.

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